



Analysis of Growth and Physiological Characteristics of Maize under Salinity Stress and its Mitigation by Seed Priming with Thiourea

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ABSTRACT

Background: Among the many abiotic stresses, salinity stress is the most pervasive one. It affects plant productivity by altering the physiological phenomena. The seed priming strategy has proved of great significance in reducing the salinity stress effects. Thiourea has been least investigated for its specific physiological effects in improving salinity stress tolerance in plants.

Objectives: The purpose of this study was to explore the role of thiourea seed priming on the physiological and biochemical basis of salinity tolerance in hybrid maize (*Zea mays* L.) at early growth stage.

Methodology: The seed of maize hybrid Ujala were unsoaked, soaked in water (hydro-primed) and 400 μ M thiourea (thiourea primed) and sown in plastic pots containing 5 kg of loam soil. After 28 days of growth, the increased salinity treatment (0, 50, 75 and 100 mM) were applied in installments of 25 mM per day. The plants were grown for 24 days after salinity treatment application. The data were recorded for different morphological and biochemical parameters using standard analytical procedures.

Results: Salinity stress adversely affected both physiological and growth attributes due to causing oxidative damage to cellular membranes, ion-toxicity and induced water deficit. However, thiourea primed plants manifested greater salinity tolerance in maize as compared to control and hydro-primed plants by showing improvements in photosynthetic pigments, osmoprotectants, vitamins and phenolic compounds. This was due to the defined role of thiourea to reduce oxidative damage and provide nitrogen nutrition by virtue of the presence of thiol, amino and imino groups in its molecule.

Conclusion: Seed priming with thiourea emerged to be an effective strategy for alleviating salinity stress in hybrid maize due to biological effects. The results suggested that thiourea seed priming can be effectively used to obtain improved maize growth in marginally to moderately salinity-hit areas.

INTRODUCTION

During growth and development, plants face numerous abiotic factors. In semiarid and arid areas of the world, soil salinity is the main factor reducing crop production is soil salinity. High salinity stress results in a random loss of crops production (Aryal *et al.* 2025). Seed germination or seedling production is also delayed or completely inhibited due to ion-toxicity and osmotic stress. Salinity induced osmotic stress affects the germination of seeds by reducing the

availability of water thereby causing ion toxicity due to greater amount of ions on a per unit basis (Manono *et al.* 2026). The plants show closely related responses to the salinity and drought stress, because changes in physiological processes are due to deficiency in the uptake of water (Tavili *et al.* 2011). Changes in the metabolism of plants lead to oxidative stresses, resulting in the loss of true plant performance (Hasanuzzaman *et al.* 2021). In maize under salinity stress, the antioxidants and osmolytes help in salinity stress tolerance. Various defense mechanisms such

as hormonal signaling pathways activation controlled by abscisic acid, ethylene, jasmonic acid, and salicylic acid and reactive oxygen species signaling pathways are activated due to abiotic stress (Fujita *et al.* 2006).

In Pakistan, maize (*Zea mays* L.) ranks third most important crop in the cropping system after wheat and rice. It is also known as 'king of the grain crops' as it gives greater grain yield. This crop is widely cultivated in the world and gives more than 50% calories of energy (Ranum *et al.* 2014). Chemical analysis shows that maize grain has 3.0% sugar, 4.8% oil, 5.8% fiber, 72% starch and 10% proteins due to which it is used to manufacture a large number of products and also used as fodder for the livestock (Hanif and Akhtar 2020).

Exogenous application to promote crop yield is a fascinating field of research (Jamei *et al.* 2026). Seed priming with synthetic or natural compounds done before the seed sowing minimizes the biotic and abiotic stress. It protects the plants against stresses without affecting the fitness of plants (Van Hulten *et al.* 2006). Priming of seeds increases the quality of seeds e.g., primed seeds enhanced the germination rate by increasing the uptake of water and rapidly resuming the metabolism rate (Paparella *et al.* 2015). The primed seeds have high percentage of total germination rate, high uniformity and increase in germination rate (Hussain *et al.* 2026). It has an ability to increase the viability and sustainability of seeds (Butler *et al.* 2009).

Different techniques of priming such as hydropriming increases seedling sustainability, crop production and capacity of seeds towards the osmotic adjustment under stress conditions (Abebe and Modi 2009). Under the drought conditions hydro-primed seeds give 3–4 times more increase in shoot and root growth as compared to the non-primed seeds (Adhikari *et al.* 2024). Hydropriming increases the biochemical and physiological processes in seeds even if there is a lowest matric potential and osmotic potential in the medium (Hussain *et al.* 2026).

Thiourea, chemically called Thiocarbamide, is an important compound with great biological effects. The presence of two functional group viz., amino (-NH₂) and thiol (-SH) in thiourea which makes it biologically more compatible. Thiol group in thiourea stabilizes the chemistry of protein's groups while -NH₂ has a lone pair of electrons due to which it acts as a base (Shanu *et al.* 2013). Recent studies showed that thiourea increases the growth rate in the entire plant along with growth at cellular level under salt stress (Granaz *et al.* 2022). It is reported that 1.3 mM thiourea overcame the salinity and cold stresses and improved the germination potential in *Capsicum* seed (Yadav *et al.* 2011). The germination characters such as mean germination time, rate and final percentage etc. improved due to soaking of *Berberis jaschakaena* seed in different priming agents for 72 h (Belwal *et al.* 2015).

As soil salinity is a great abiotic stress factor for field crops, there was a need to find strategies to combat this adversary. Among the many chemicals used, exogenous use

of thiourea has been widely used to improve growth of various plant species under stressful conditions (Wahid *et al.* 2017), however clear mechanism of action is elusive yet. In the present study, thiourea was used as seed priming agent to determine its effectiveness under increased salt stress in a controlled pot experiment by measuring some growth and physiological attributes.

MATERIALS AND METHODS

Experimental details

For the present study, the F1 seeds of maize (*Zea mays* L.) hybrid Ujala were kindly provided by Sohni Dharti Seed Company, Sahiwal, Punjab. Thiourea and pure sodium chloride (NaCl) were purchased from Merck. Distilled water was used for preparation of thiourea priming solution and salt solutions. The experiment was conducted in the Old Botanical Garden of the University of Agriculture, Faisalabad, Pakistan. The soil used to grow the plants was loam. Before sowing the pH and EC_e of the soil was determined as 8.13 and 1.5 ds/m. The organic matter content was 40% and water holding capacity was 55%.

Treatment application

Healthy looking seeds of uniform size were used to apply priming treatments. The priming with water (hydropriming) and 400 µM thiourea (thiourea-priming) was done by soaking seeds for 8 h, washed with distilled water and sown in the potted soil. Prior to filling soil in the pots, a hole was made in the bottom of the pot and a piece of muslin cloth was kept in the hole to avoid excessive loss of soil solution. Hydro- and, thiourea-primed and unprimed seeds were sown in separate plastic pots for each priming and salt treatments. Pots were arranged in completely randomized manner with three replications, one hybrids variety ujala, two priming treatments, one treatment is without priming or unsoaked, and three levels of salinity (0 control, 50, 75, 100 mM). The plants were allowed to grow under priming treatments for 28 days then salinity stress applied on plants in installments of 25 mM for four days up to the level of 100 mM. Harvesting was done after 24 days of salinity stress applications.

Sampling for plant analysis

Immediately after harvest, one half of the shoot and root samples from each replicate and treatment were or further biochemical analysis fresh plant sample were placed in the zipper bags, properly labeled and kept in a freezer at -40°C. The other half was put in paper bags and dried in an oven at 65°C for one week and used for dry analysis.

Data collection

Growth attributes: After uprooting the plants and briefly washing in running tap water and blot drying, shoots were

separated from roots. The morphological data were recorded for no. of leaves per plant, fresh and dry weight of shoot and root.

Pigment contents: Chlorophyll a and chlorophyll b were estimated by using the method of Arnon (1949). The carotenoids contents were determined by using the method of Davies (1976). For this purpose, 0.1 g plant leaf sample was grinded in 2 mL of 80% acetone. The volume was maintained up to 10 mL by adding 80% acetone. The absorption of the extract was taken at 663, 645 and 480 nm using spectrophotometer (UV-3802, UNIC, Shanghai, China). The pigment contents were calculated by the following formulas:

$$\text{Chl. a (mg mL}^{-1}\text{)} = [12.7(\text{OD } 663) - 2.69(\text{OD } 645)] \times V/1000 \times W/28$$

$$\text{Chl. b (mg mL}^{-1}\text{)} = [22.9(\text{OD } 645) - 4.68(\text{OD } 663)] \times V/1000 \times W$$

Where V = Volume of the extract (mL), W = weight of the fresh leaf (g)

$$\text{Carotenoids} = [(OD \ 480) + 0.114(OD \ 663) - 0.638(OD \ 645)] \times E \ 100\% \text{ cm} = 2500$$

$$\text{Carotenoids (mg mL}^{-1}\text{)} = \text{Acar} / \text{Em} 100\% \times 10$$

Phenolics analysis: Fresh material (0.1 g) was extracted using 80% acetone, which was used for analysis of different phenolic compounds. Julkunen-Tiitto (1985) method was used for spectrophotometric analysis of soluble phenolics at 730 nm. Flavonoid content was measured spectrophotometrically at 590 nm according to the method of Zhishen *et al.* (1999). Method of Stark and Wray (1989) was followed for anthocyanin analysis using spectrophotometer at 510 nm.

Ionic analysis: Ionic contents were determined by digesting the dried plant sample in 2 mL of hydrogen peroxide (H₂O₂) and 5–7 mL of concentrated nitric acid (HNO₃) on the hot plate at 250°C. This solution was digested up to 50 mL and Na⁺, K⁺ and Ca²⁺ were determined using flame photometer.

Statistical analysis

Computer software “Statistix 8.1” was used for all statistical analysis and MS office excel sheets were used to represent the data of three replicates in graphical form. By using the analysis of variance (ANOVA) significant or non-significant differences between the parameters were determined using LSD test at 5% probability level.

RESULTS

Morphological attributes

The statistical analysis of data revealed that there was a non-significant interaction ($P > 0.05$) between the priming and salinity stress treatments (Table 1). Priming alleviated the adverse effect of salinity stress in shoot and root length, dry weight, no. of leaves per plant and leaf area per plant. Results showed that increased salinity levels decreased all these morphological characteristics but the priming with 400 μM thiourea solution significantly improved these attributes both under control and salinity stress. More specifically, the leaf area per plant was distinctly reduced by applied salinity

but greatly improved in thiourea primed set exposed to salinity stress (Fig. 1).

Photosynthetic pigment contents

Statistical analysis of data for chlorophyll a, b and carotenoids a non-significant interaction ($P > 0.05$) between the priming and salinity stress while there was a highly significant interaction ($P < 0.01$) observed in carotenoids contents (Table 1). Results showed that salinity stress decreased the photosynthetic pigment contents. As compared to unsoaked group of plants, thiourea and water primed plants improved the photosynthetic pigment contents, especially the carotenoids under both control and salinity stress conditions (Fig. 2).

Phenolic compounds

Statistical analysis of data showed significant ($P < 0.01$) difference in priming and salinity treatments in all the phenolics including soluble phenolics, flavonoids and anthocyanins with significant interaction of salinity and priming treatments for shoot phenolics ($P < 0.05$), root flavonoid ($P < 0.01$) and root anthocyanins ($P < 0.01$) (Table 1). Perusal of data revealed that soluble phenolics and anthocyanins were lowest under control conditions, which increased with increased salinity levels. However, water and thiourea priming further increased their levels both in shoot and root (Fig. 3). On the other hand, shoot and root flavonoids were higher under control but decreased as the salinity levels increased. However, thiourea followed by water priming were effective in increasing shoot and root flavonoids under the highest salinity level (Fig. 3).

Ionic contents analysis

Statistical analysis of data for shoot and root Na⁺, K⁺ and Ca²⁺ in the shoot and root of thiourea primed, water primed and unsoaked plants indicated significant ($P < 0.01$) difference with a significant ($P < 0.01$) interaction of these factors for root Na⁺ and root Ca²⁺ (Table 1). Graphical data showed that Na⁺ increased with increase in the salinity levels while K⁺ and Ca²⁺ contents decreased with increase in the salinity levels but the thiourea priming alleviated the salinity stress both in the shoot and root tissues than water primed or non-primed plants (Table 2).

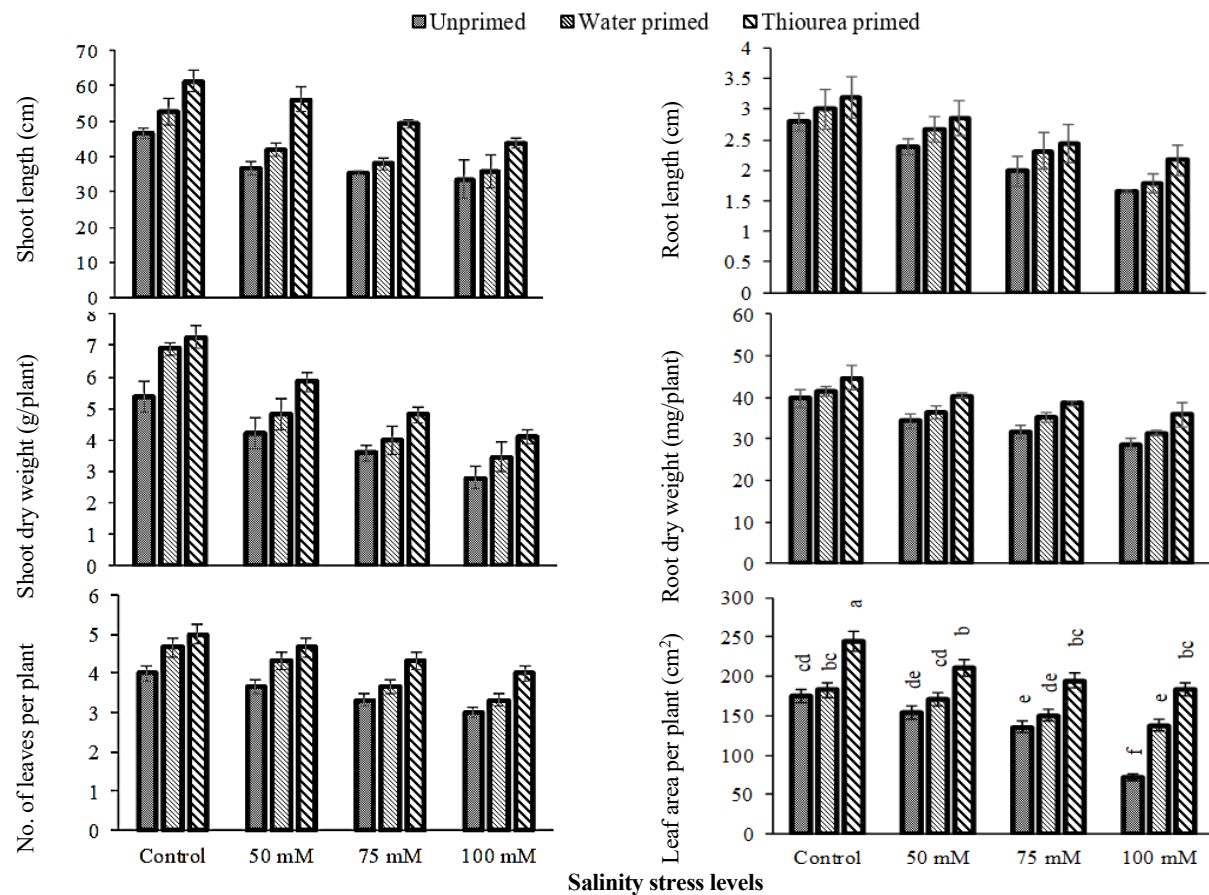
DISCUSSION

Salinity is one of the major abiotic factors that highly reduces crop production each year. Salinity adversely affects the most sensitive stages of crop establishment e.g., seed germination and seedling growth. Hence, salinity stress is the major problem for crop yield (Patade *et al.* 2011). Studies show that seed priming is one of the most important techniques used to improve seed germination under saline

Table 1: Analysis of variance (mean squares) of the applied salinity and priming treatments and their interactions for different parameters of maize

Parameters	Sources of variation			
	Priming (Pr)	Salinity (Sal)	Pr×Sal	Error
Shoot length	676.090**	422.944**	13.674ns	8.819
Root length	120.424*	155.025**	1.053ns	21.875
Shoot dry weight	6.766**	15.596**	0.218ns	0.220
Root dry weight	0.292*	2.132**	0.028ns	0.062
No. of leaves per plant	3.000**	2.148**	0.037ns	0.222
Leaf area per plant	16817.100**	7829.700**	747.800*	282.900
Chlorophyll a	0.477**	0.421**	0.047ns	0.035
Chlorophyll b	0.523**	0.295**	0.012ns	0.011
Carotenoids	0.415**	0.343**	0.042**	0.009
Shoot soluble phenolics	0.259**	0.532**	0.004*	0.001
Root soluble phenolics	0.121**	0.358**	0.002ns	0.001
Shoot flavonoids	0.280**	0.404**	0.004ns	0.004
Root flavonoids	0.270**	0.316**	0.006**	0.002
Shoot anthocyanins	0.872**	0.355**	0.008ns	0.008
Root anthocyanins	0.377**	0.480**	0.005*	0.002
Shoot Na ⁺	3512.450**	224.333**	24.280**	3.044
Root Na ⁺	178.420**	4337.340**	41.170**	2.181
Shoot K ⁺	42.966**	663.457**	0.993ns	0.997
Root K ⁺	3.366**	276.059**	1.220ns	0.509
Shoot Ca ²⁺	0.928**	62.013**	0.186ns	0.102
Root Ca ²⁺	0.349**	11.652**	0.025*	0.063

Significant at: ** P<0.01, * P<0.05 and NS non-significant. Error degree of freedom 24

**Fig. 1:** Changes in growth parameters of maize plants grown from unprimed, hydro-primed and thiourea-primed seeds under increased salinity levels. Different letters on the data points indicate significant (P<0.05) differences among them.

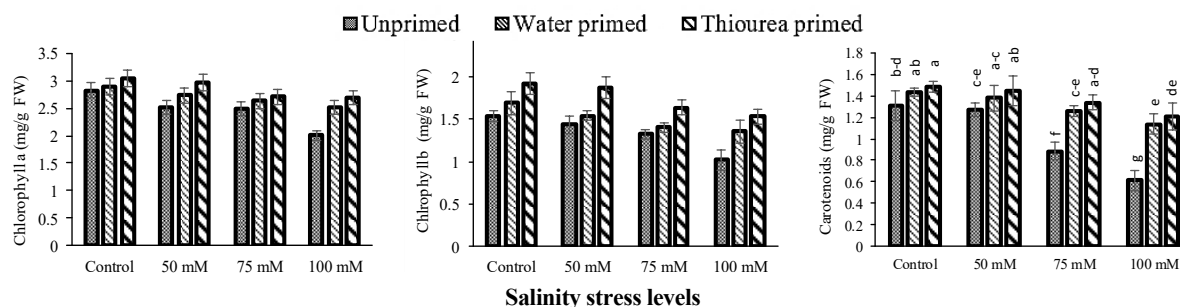


Fig. 2: Changes in photosynthetic pigment contents of maize plants raised from unprimed, hydro-primed and thiourea-primed seeds under increased salinity levels. Different letters on the data points indicate significant ($P < 0.05$) differences among them.

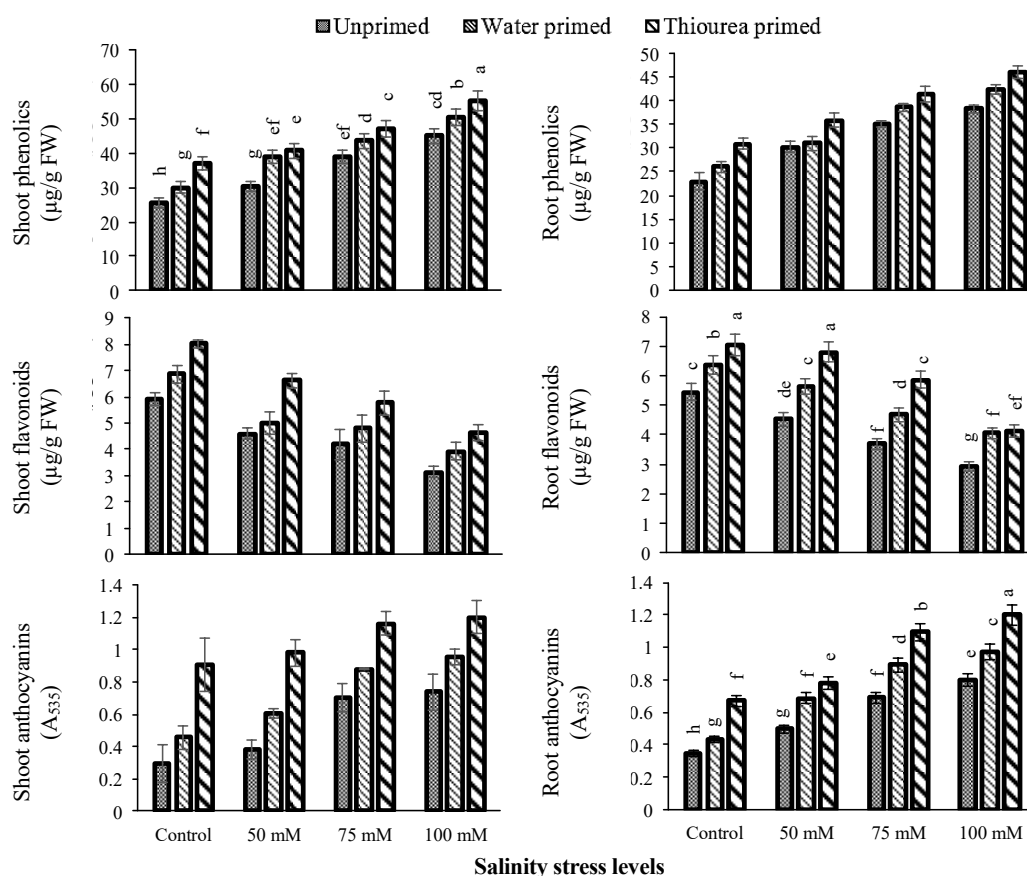


Fig. 3: Changes in phenolics concentration in shoot and root of maize plants grown from unprimed, hydro-primed and thiourea-primed seeds under increased salinity levels. Different letters on the data points indicate significant ($P < 0.05$) differences among them.

conditions. Different natural and synthetic compounds were used before seed germination. Primed plants showed quick and sturdy cellular defenses against abiotic stresses (Biswas *et al.* 2023; Jamei *et al.* 2026).

Application of salinity stress at different levels resulted in decreased in shoot and root length, dry weight, number of leaves and leaf area per plant while thiourea priming was capable to reduce the salt related adversary

on maize to remarkable limits (Table 1; Fig. 1). It is reported that salinity-induced reduction in plant growth can be successfully nullified by the exogenous application of stress alleviating agents (Mahajan and Tuteja 2005; Granaz *et al.* 2022), and the same was substantiated in this study by seed priming with thiourea. This has great implications for improving plant yield under salinity stress.

Table 2: Ionic concentrations in the shoot and root tissues of maize plants grown from unprimed (control), hydro-primed and thiourea-primed

NaCl Salinity stress	Priming	Shoot Na ⁺	Root Na ⁺	Shoot K ⁺	Root K ⁺	Shoot Ca ²⁺	Root Ca ²⁺
Concentration (mg/g dry weight)							
Control	Control	5.20±0.29g	8.82±0.52g	31.64±0.89a	22.94±1.37a	10.25±0.45a	6.78±0.46ab
	Hydropriming	5.38±0.24g	8.76±0.56g	32.67±1.24a	22.45±0.66a	10.57±0.57a	6.81±0.22a
	Thiourea priming	5.34±0.19g	8.57±0.41g	33.99±1.31a	22.65±0.46a	11.46±0.27a	6.97±0.23a
50 mM	Control	21.90±1.65e	26.50±1.26e	19.98±1.25a	15.37±0.84a	7.30±0.28a	6.23±0.21c
	Water	20.38±0.89ef	26.42±0.94e	20.25±1.30a	15.04±0.62a	7.25±0.29a	6.37±0.27bc
	Thiourea	18.69±1.27f	22.18±1.88f	24.09±1.33a	15.64±0.54a	7.74±0.45a	6.71±0.35ab
75 mM	Control	37.28±1.67c	45.87±1.89c	16.24±0.53a	11.37±0.71a	5.69±0.30a	4.88±0.13de
	Water	34.58±1.50c	44.57±0.83c	16.87±0.66a	11.41±0.96a	5.73±0.24a	5.16±0.25d
	Thiourea	30.53±2.07d	38.43±1.49d	20.27±0.82a	13.05±0.73a	5.86±0.22a	5.17±0.18d
100 mM	Control	56.08±3.95a	64.98±2.25a	10.96±0.47a	8.96±0.19a	4.62±0.19a	4.27±0.17f
	Water	54.65±1.88a	64.08±2.13a	11.80±0.67a	10.27±0.17a	4.79±0.10a	4.35±0.19f
	Thiourea	44.22±1.64b	49.18±1.83b	14.73±0.78a	11.21±0.42a	4.92±0.14a	4.66±0.17ef

Letters with same alphabet differ non-significantly (P>0.05)

Salinity induced changes observed in plants and their potential reversal with the exogenous applications are due to some important physiological changes taking place in metabolic pathways, which is important to explore to find the possible reasons for improved plant growth. In this study, there was a marked improvement in the chlorophyll a, b and carotenoids with thiourea priming (Fig. 2), which was due to reduction in ion-toxicity and increase in beneficial nutrients (K⁺ and Ca²⁺). Turan (2009) described that decrease in plant growth may be due to excessive accumulation of ions or osmotic reduction in water availability. Perveen *et al.* (2013) reported that higher level of thiourea showed inhibition in growth while 0.25 mM thiourea applied to the maize which is grown in roots is highly effective for total dry mass and for root growth and health. Mehmood *et al.* (2021) described that the most effective range of thiourea level was 400 µM, which played a major role in hybrid maize explant for the origination of root and shoot.

Among the photosynthetic pigments, carotenoid levels were specifically improved with thiourea priming, which indicated that thiourea plays a role in improving photosynthesis in maize leaves (Fig. 2). In fact, carotenoids play dual roles of harvesting light for use in photosynthesis and prevent photooxidative damage to chloroplastic lamellae (Simkin *et al.* 2022). In this context, Cha-um and Kirdamanee (2009) reported that chlorophyll concentration was reduced by salinity stress. Chlorophyll fluorescence data has revealed that photon yield decreases due to increase in the salinity stress, which results in a considerable reduction in the plant growth and development (Kalaji *et al.* 2011; Hosseini *et al.* 2023).

Wang *et al.* (2024) reported that due salinity stress reactive oxygen species produced that causes oxidation of cellular membrane and photosynthetic pigments by damaging the chloroplastic membranes. Parveen *et al.* (2018) described that on maize plant addition of 0.25 mM thiourea results in the biosynthesis of antioxidants such as anthocyanins, phenols, ascorbic acid. Mohamed *et al.*

(2018) reported that in maize, the levels of antioxidants and osmoprotectants were adversely affected by the soil salinity. They reported that as compared to control plants, there was a reduction in flavonoid contents due to salinity stress. In this study, although applied salinity increased the contents of soluble phenolics, flavonoids and anthocyanins as salt stress response, however their levels were further enhanced with thiourea priming (Fig. 3). These findings indicated an adaptive role of thiourea in improving salinity stress resistance in maize. The phenolic compounds have been considered pivotal in improving salinity tolerance in different plant species (Ahlawat *et al.* 2024; Gul *et al.* 2024). As reported, salinity stress resistance properties of phenolics compounds appear to be due to the presence of hydroxyl group attached to benzene ring structure, which detoxify the harmful ions and ROS produced under salinity stress (Michalak 2015; Šamec *et al.* 2021).

CONCLUSION

Salinity stress decreased all growth parameters, increased some secondary metabolites and sodium ions contents. Priming especially thiourea priming was found effective in reducing the toxic effect of salinity by improving all ionic, growth, biochemical and physiological parameters. The alleviation of salt stress effect on maize appears to be due to the structural properties of thiourea molecule, which are pivotal in detoxification of the reactive oxygen species and excess ions in the shoot and root tissues.

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DATA AVAILABILITY

The data will be made available on a fair request.

ETHICS APPROVAL

Not applicable to this study.

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